Purified cultures of mouse embryonic stem cell-derived cardiomyocytes for electrophysiological studies with ionising radiation*

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High doses of ionising radiation to the heart are known to induce late-occurring ischemic heart disease [1] and epidemiological data suggest an increased risk of cardiovascular diseases also for an exposure to low doses [2]. To investigate putative adverse effects of ionising radiation on the electrophysiology of the heart, we recently set up a model system using mouse embryonic stem cell (mESC)-derived cardiomyocytes [3].

Briefly, this system is based on the differentiation of pluripotent stem cells through formation of embryoid bodies (EBs) [4]. EBs comprise a variety of different cell types, among them spontaneously contracting cardiomyocytes. For electrophysiological studies, EBs were seeded on the electrode array of a microelectrode array chip (MEA) and measurements were conducted. Data were analysed using the MATLAB based software *DrCell* developed at University of Applied Sciences Aschaffenburg [5]. Endpoints such as signal amplitude and shape, beat rate and conduction velocity were examined. This system, however, proved to be unstable. Large intra and inter-sample variations were observed most likely resulting from the progressive differentiation of cells in the embryoid bodies. Moreover, the small number and size of spontaneously contracting clusters (i.e. cardiomyocytes) renders this model system unsuitable for the detection of the anticipated small effects of IR on the electrophysiology of cardiomyocytes [3].

To overcome these drawbacks, we subsequently used a commercially available purified culture of mESC-derived cardiomyocytes (Cor.At, Axiogenesis, Fig. 1). Cor.At cells were seeded on the fibronectin coated electrode array of a MEA according to the manufacturer's protocol. Measurements were conducted at 24 h intervals and subsequently analysed with DrCell software.

Figures 2 and 3 exemplarily show data for two of the analysed endpoints, i.e. the number of active electrodes and the beat rate of control samples (n=35) of three different experiments. Regarding sample variations, purified Cor.At cardiomyocytes are more stable than contracting cardiomyocytes generated within an EB. Concerning the number of active electrodes (Fig. 2) larger variations are observed at day 1 and 2 in culture due to the recovery process after thawing and the time needed to build up a conducting network. At days 8 and 9 in culture, Cor.At cells start to detach from the MEA surface leading to higher variations in the number of active electrodes. However, from day 2 until day 7 the number of active electrodes is stable, reflected in small error bars. As shown in figure 3, from day 2 on the beat rate increases steadily with a considerably smaller inter-sample variations when compared to the number of active electrodes. Altogether our studies show that purified Cor.At cells are a suitable tool to investigate the effect of ionising radiation on the electrophysiological response of cardiomyocytes. Based on these data, x-ray experiments have been conducted and are currently analysed.

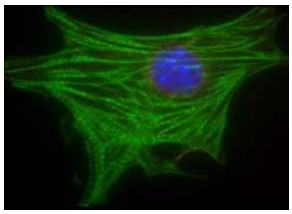

Figure 1: Representative immunofluorescence image of a Cor.At cardiomyocyte (red: connexin 43; green: cardiac-specific protein Troponin; blue: DNA).

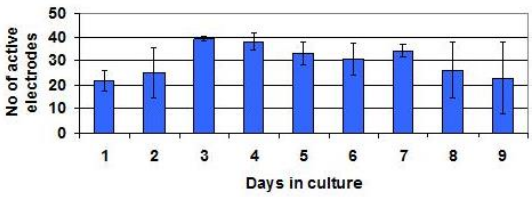

Figure 2: Number of active electrodes. Measurements were performed on control samples of three different experiments (total n=35, SEM).

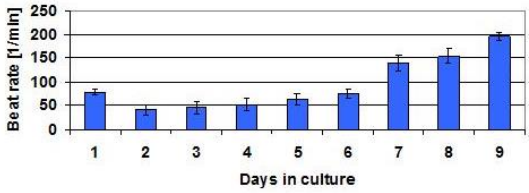

Figure 3: Beat rate. Measurements were performed on control samples of three different experiments (total n=35, SEM).

References

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